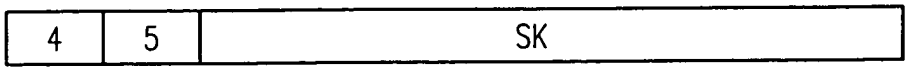
(A) FBD(1,2) fused at the C-terminal of SK

(B) FBD(4,5) fused at the C-terminal of SK

(C) FBD(4,5) fused at the N-terminal of SK



(D) FBD(4,5) fused at both the C as well as N-terminals of SK

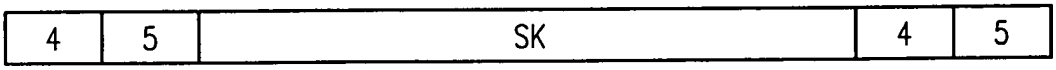


FIG. 1

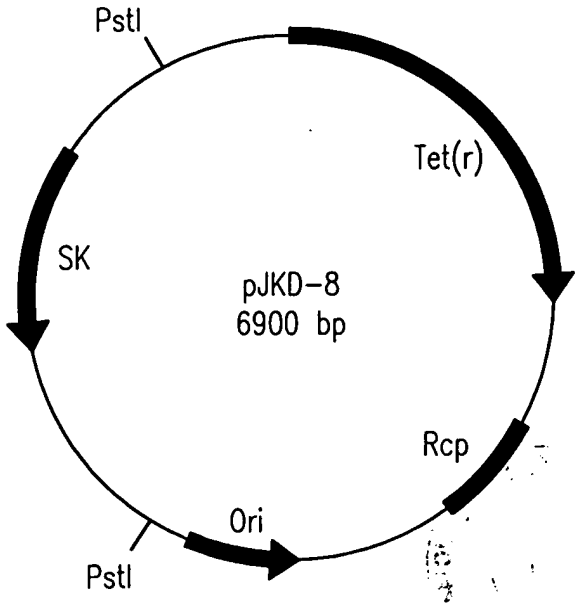


FIG. 2

FIG. 3-1

```
1/1                                                 31/11
ATT GCT GGA CCT GAG TGG CTG CTA GAC CGT CCA TCT GTC AAC AAC AGC CAA TTA GTT GTT
ile ala gly pro glu trp leu leu asp arg pro ser val asn asn ser gln leu val val
61/21                                               91/31
AGC GTT GCT GGT ACT GTT GAG GGG ACG AAT CAA GAC ATT AGT CTT AAA TTT TTT GAA ATC
ser val ala gly thr val glu gly thr asn gln asp ile ser leu lys phe phe glu ile
121/41                                              151/51
GAT CTA ACA TCA CGA CCT GCT CAT GGA GGA AAG ACA GAG CAA GGC TTA AGT CCA AAA TCA
asp leu thr ser arg pro ala his gly gly lys thr glu gln gly leu ser pro lys ser
181/61                                              211/71
AAA CCA TTT GCT ACT GAT AGT GGC GCG ATG TCA CAT AAA CTT GAG AAA GCT GAC TTA CTA
lys pro phe ala thr asp ser gly ala met ser his lys leu glu lys ala asp leu leu
241/81                                              271/91
AAG GCT ATT CAA GAA CAA TTG ATC GCT AAC GTC CAC AGT AAC GAC GAC TAC TTT GAG GTC
lys ala ile gln glu gln leu ile ala asn val his ser asn asp asp tyr phe glu val
301/101                                             331/111
ATT GAT TTT GCA AGC GAT GCA ACC ATT ACT GAT CGA AAC GGC AAG GTC TAC TTT GCT GAC
ile asp phe ala ser asp ala thr ile thr asp arg asn gly lys val tyr phe ala asp
361/121                                             391/131
AAA GAT GGT TCG GTA ACC TTG CCG ACC CAA CCT GTC CAA GAA TTT TTG CTA AGC GGA CAT
lys asp gly ser val thr leu pro thr gln pro val gln glu phe leu leu ser gly his
421/141                                             451/151
GTG CGC GTT AGA CCA TAT AAA GAA AAA CCA ATA CAA AAC CAA GCG AAA TCT GTT GAT GTG
val arg val arg pro tyr lys glu lys pro ile gln asn gln ala lys ser val asp val
481/161                                             511/171
GAA TAT ACT GTA CAG TTT ACT CCC TTA AAC CCT GAT GAC GAT TTC AGA CCA GGT CTC AAA
glu tyr thr val gln phe thr pro leu asn pro asp asp asp phe arg pro gly leu lys
541/181                                             571/191
GAT ACT AAG CTA TTG AAA ACA CTA GCT ATC GGT GAC ACC ATC ACA TCT CAA GAA TTA CTA
asp thr lys leu leu lys thr leu ala ile gly asp thr ile thr ser gln glu leu leu
601/201                                             631/211
GCT CAA GCA CAA AGC ATT TTA AAC AAA AAC CAC CCA GGC TAT ACG ATT TAT GAA CGT GAC
ala gln ala gln ser ile leu asn lys asn his pro gly tyr thr ile tyr glu arg asp
661/221                                             691/231
TCC TCA ATC GTC ACT CAT GAC AAT GAC ATT TTC CGT ACG ATT TTA CCA ATG GAT CAA GAG
ser ser ile val thr his asp asn asp ile phe arg thr ile leu pro met asp gln glu
721/241                                             751/251
TTT ACT TAC CGT GTT AAA AAT CGG GAA CAA GCT TAT AGG ATC AAT AAA AAA TCT GGT CTG
phe thr tyr arg val lys asn arg glu gln ala tyr arg ile asn lys lys ser gly leu
781/261                                             811/271
AAT CAA GAA ATA AAC AAC ACT GAC CTG ATC TCT GAG AAA TAT TAC GTC CTT AAA AAA GGG
asn glu glu ile asn asn thr asp leu ile ser glu lys tyr tyr val leu lys lys gly
841/281                                             871/291
GAA AAG CCG TAT GAT CCC TTT GAT CGC AGT CAC TTG AAA CTG TTC ACC ATC AAA TAC GTT
glu lys pro tyr asp pro phe asp arg ser his leu lys leu phe thr ile lys tyr val
901/301                                             931/311
GAT GTC GAT ACC AAC GAA TTG CTA AAA AGT GAG CAG CTC TTA ACA GCT AGC GAA CGT AAC
asp val asp thr asn glu leu leu lys ser glu gln leu leu thr ala ser glu arg asn
```

FIG. 3-2

```
961/321                                          991/331
TTA GAC TTC AGA GAT TTA TAC GAT CCT CGT GAT AAG GCT AAA CTA CTC TAC AAC AAT CTC
leu asp phe arg asp leu tyr asp pro arg asp lys ala lys leu leu tyr asn asn leu
1021/341                                         1051/351
GAT GCT TTT GGT ATT ATG GAC TAT ACC TTA ACT GGA AAA GTA GAG GAT AAT CAC GAT GAC
asp ala phe gly ile met asp tyr thr leu thr gly lys val glu asp asn his asp asp
1081/361                                         1111/371
ACC AAC CGT ATC ATA ACC GTT TAT ATG GGC AAG CGA CCC GAA GGA GAG AAT GCT AGC TAT
thr asn arg ile ile thr val tyr met gly lys arg pro glu gly glu asn ala ser tyr
1141/381                                         1171/391
CAT TTA GCC TAT GAT AAA GAT CGT TAT ACC GAA GAA GAA CGA GAA GTT TAC AGC TAC CTG
his leu ala tyr asp lys asp arg tyr thr glu glu glu arg glu val tyr ser tyr leu
1201/401                                         1231/411
CGT TAT ACA GGG ACA CCT ATA CCT GAT AAC CCT AAC GAC AAA TAA
arg tyr thr gly thr pro ile pro asp asn pro asn asp lys OCH
```

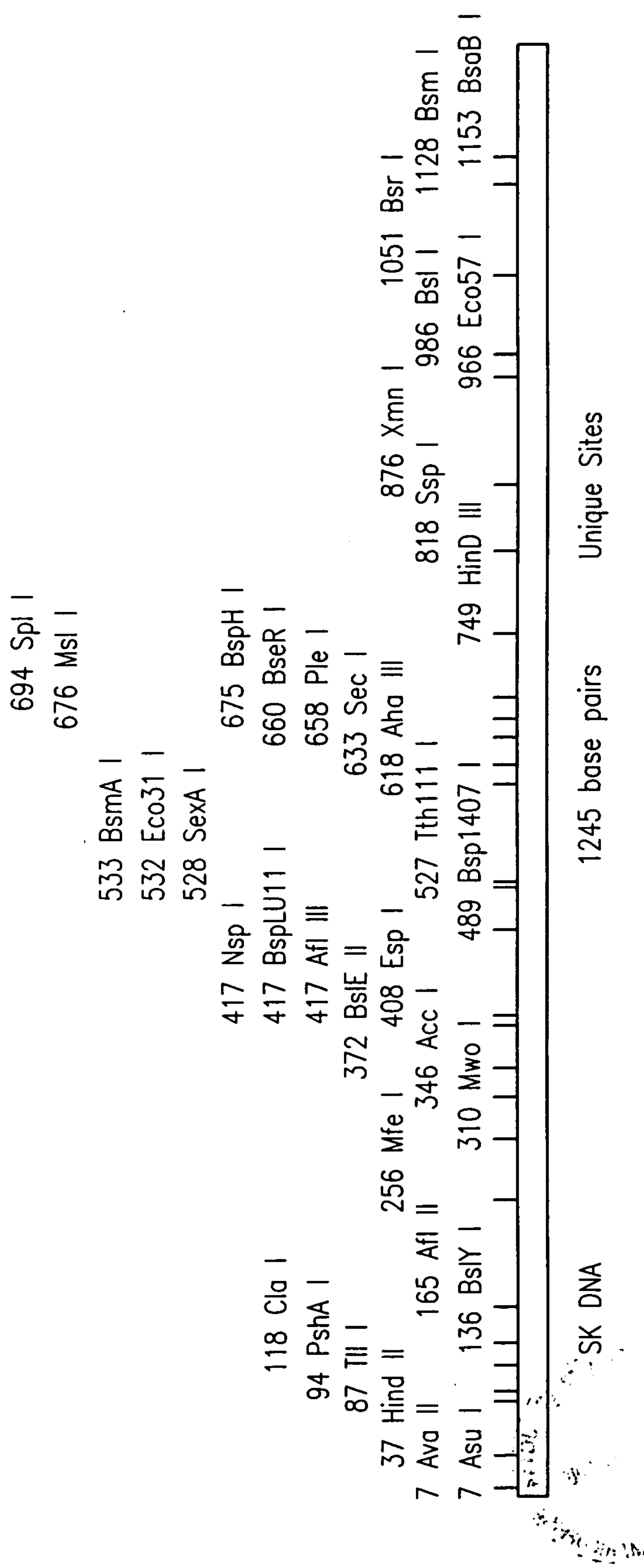

7 Asu I
7 Ava II
37 Hind II
87 Tll I
94 PshA I
118 Cla I
136 BslY I
165 Afl II
256 Mfe I
310 Mwo I
346 Acc I
372 BslE II
408 Esp I
417 Afl III
417 BspLU11 I
417 Nsp I
489 Bsp1407 I
527 Tth111 I
528 SexA I
532 Eco31 I
533 BsmA I
618 Aha III
633 Sec I
658 Ple I
660 BseR I
675 BspH I
676 Msl I
694 Spl I
749 HinD III
818 Ssp I
876 Xmn I
966 Eco57 I
986 Bsl I
1051 Bsr I
1128 Bsm I
1153 BsaB I
SK DNA
1245 base pairs
Unique Sites

FIG. 4

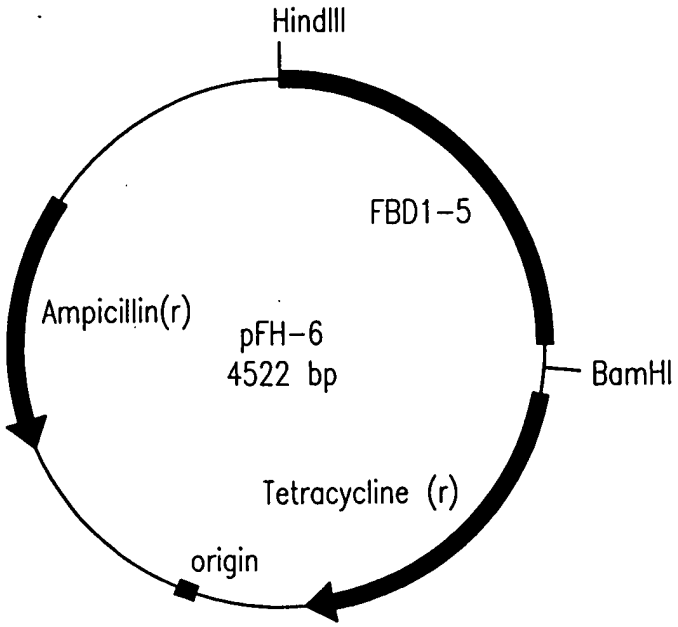

HindIII
FBD1-5
Ampicillin(r)
pFH-6
4522 bp
BamHI
Tetracycline (r)
origin

FIG. 5

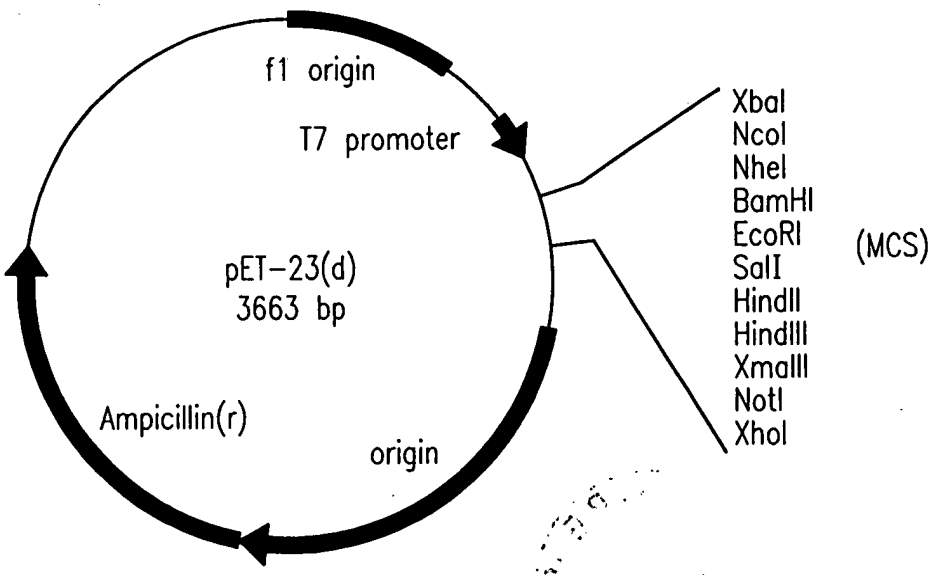

f1 origin
T7 promoter
pET-23(d)
3663 bp
Ampicillin(r)
origin
XbaI
NcoI
NheI
BamHI
EcoRI
SalI
HindII
HindIII
XmaIII
NotI
XhoI
(MCS)

FIG. 8

FIG. 6

```
1/1                                                 31/11
CAG GCT CAG CAA ATG GTT CAG CCC CAG TCC CCG GTG GCT GTC ACT CAA AGC AAG CCC GGT
gln ala gln gln met val gln pro gln ser pro val ala val ser gln ser lys pro gly
61/21                                               91/31
TGT TAT GAC AAT GGA AAA CAC TAT CAG ATA AAT CAA CAG TGG GAG CGG ACC TAC CTA GGT
cys tyr asp asn gly lys his tyr gln ile asn gln gln trp glu arg thr tyr leu gly
121/41                                              151/51
AAT GTG TTG GTT TGT ACT TGT TAT GGA GGA AGC CGA GGT TTT AAC TGC GAA AGT AAA CCT
asn val leu val cys thr cys tyr gly gly ser arg gly phe asn cys glu ser lys pro
181/61                                              211/71
GAA GCT GAA GAG ACT TGC TTT GAC AAG TAC ACT GGG AAC ACT TAC CGA GTG GGT GAC ACT
glu ala glu glu thr cys phe asp lys tyr thr gly asn thr tyr arg val gly asp thr
241/81                                              271/91
TAT GAG CGT CCT AAA GAC TCC ATG ATC TGG GAC TGT ACC TGC ATC GGG GCT GGG CGA GGG
tyr glu arg pro lys asp ser met ile trp asp cys thr cys ile gly ala gly arg gly
301/101                                             331/111
AGA ATA AGC TGT ACC ATC GCA AAC CGC TGC CAT GAA GGG GGT CAG TCC TAC AAG ATT GGT
arg ile ser cys thr ile ala asn arg cys his glu gly gly gln ser tyr lys ile gly
361/121                                             391/131
GAC ACC TGG AGG AGA CCA CAT GAG ACT GGT GGT TAC ATG TTA GAG TGT GTG TGT CTT GGT
asp thr trp arg arg pro his glu thr gly gly tyr met leu glu cys val cys leu gly
421/141                                             451/151
AAT GGA AAA GGA GAA TGG ACC TGC AAG CCC ATA GCT GAG AAG TGT TTT GAT CAT GCT GCT
asn gly lys gly glu trp thr cys lys pro ile ala glu lys cys phe asp his ala ala
481/161                                             511/171
GGG ACT TCC TAT GTG GTC GGA GAA ACG TGG GAG AAG CCC TAC CAA GGC TGG ATG ATG GTA
gly thr ser tyr val val gly glu thr trp glu lys pro tyr gln gly trp met met val
541/181                                             571/191
GAT TGT ACT TGC CTG GGA GAA GGC AGC GGA CGC ATC ACT TGC ACT TCT AGA AAT AGA TGC
asp cys thr cys leu gly glu gly ser gly arg ile thr cys thr ser arg asn arg cys
601/201                                             631/211
AAC GAT CAG GAC ACA AGG ACA TCC TAT AGA ATT GGA GAC ACC TGG AGC AAG AAG GAT AAT
asn asp gln asp thr arg thr ser tyr arg ile gly asp thr trp ser lys lys asp asn
661/221                                             691/231
CGA GGA AAC CTG CTC CAG TGC ATC TGC ACA GGC AAC GGC CGA GGA GAG TGG AAG TGT GAG
arg gly asn leu leu gln cys ile cys thr gly asn gly arg gly glu trp lys cys glu
721/241                                             751/251
AGG CAC ACC TCT GTG CAG ACC ACA TCG AGC GGA TCT GGC CCC TTC ACC GAT GTT CGT
arg his thr ser val gln thr thr ser ser gly ser gly pro phe thr asp val arg
```

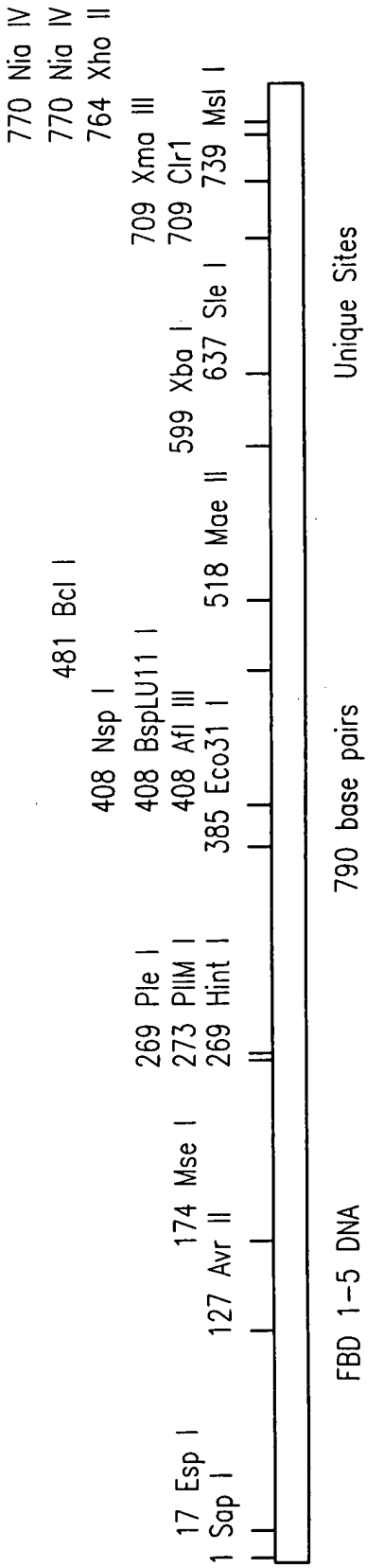

1 Sap I
17 Esp I
127 Avr II
174 Mse I
269 Hint I
269 Ple I
273 PllM I
385 Eco31 I
408 Afl III
408 BspLU11 I
408 Nsp I
481 Bcl I
518 Mae II
599 Xba I
637 Sle I
709 Clr1
709 Xma III
739 Msl I
764 Xho II
770 Nia IV
770 Nia IV
FBD 1-5 DNA
790 base pairs
Unique Sites

FIG. 7

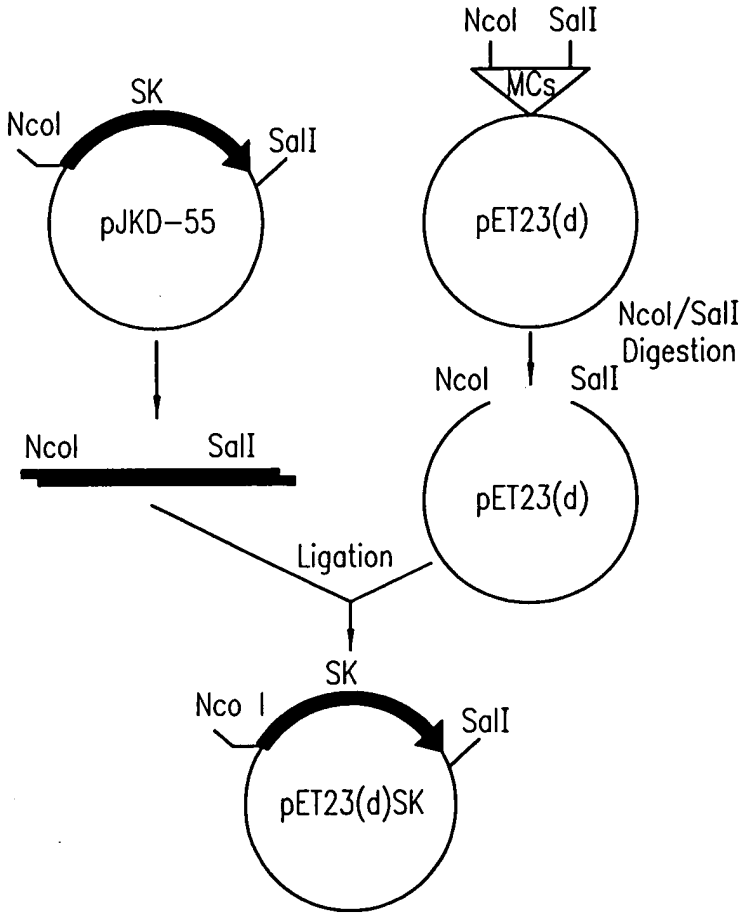

NcoI
SalI
MCs
SK
NcoI
SalI
pJKD–55
pET23(d)
NcoI/SalI
Digestion
NcoI
SalI
NcoI
SalI
pET23(d)
Ligation
SK
Nco I
SalI
pET23(d)SK

FIG. 9

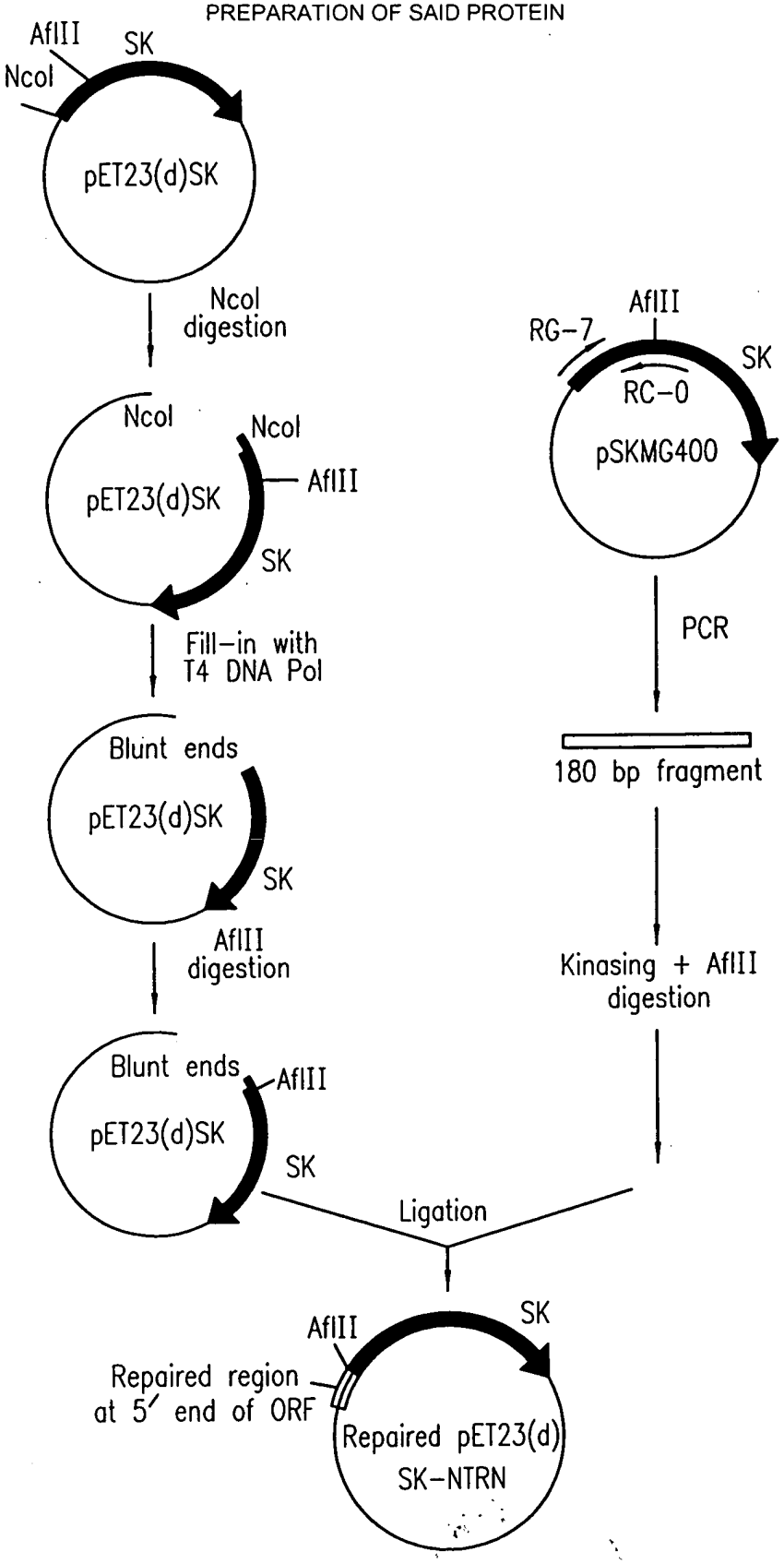

AflII
SK
NcoI
pET23(d)SK
NcoI
digestion
NcoI
NcoI
pET23(d)SK
AflII
SK
Fill-in with
T4 DNA Pol
Blunt ends
pET23(d)SK
SK
AflII
digestion
Blunt ends
AflII
pET23(d)SK
SK
AflII
RG-7
SK
RC-0
pSKMG400
PCR
180 bp fragment
Kinasing + AflII
digestion
Ligation
AflII
SK
Repaired region
at 5′ end of ORF
Repaired pET23(d)
SK-NTRN

FIG. 10

FIG. 11

	10	20	30	40	50
	GCACCCGTGG	CCAGGACCCA	ACGCTGCCCG	AGATCTCGAT	CCCGCGAAAT
51	TAATACGACT	CACTATAGGG	AGACCACAAC	GGTTTCCCTC	TAGAAATAAT
101	TTTGTTTAAC	TTTAAGAAGG	AGATATACCA	TGATTGCTGG	ACCTGAGTGG
151	CTGCTAGACC	GTCCATCTGT	CAACAACAGC	CAATTGGTTG	TTAGCGTTGC
201	TGGTACTGTT	GAGGGGACGA	ATCAAGACAT	TAGTCTTAAA	TTTTTTGAAA
251	TCGATCTAAC	ATCACGACCT	GCTCATGGAG	GAAAGACAGA	GCAAGGCTTA
301	AGTCCAAAAT	CAAAACCATT	TGCTACTGAT	AGTGGCGCGA	TGTCACATAA
351	ACTTGAGAAA	GCTGACTTAC	TAAAGGCTAT	TCAAGAACAA	TTGATCGCTA
401	ACGTCCACAG	TAACGACGAC	TACTTTGAGG	TCATTGATTT	TGCAAGCGAT
451	GCAACCATTA	CTGATCGAAA	CGGCAAGGTC	TACTTTGCTG	ACAAAGATGG
501	TTCGGTAACC	TTGCCGACCC	AACCTGTCCA	AGAATTTTTG	CTAAGCGGAC
551	ATGTGCGCGT	TAGACCATAT	AAAGAAAAAC	CAATACAAAA	CCAAGCGAAA
601	TCTGTTGATG	TGGAATATAC	TGTACAGTTT	ACTCCCTTAA	ACCCTGATGA
651	CGATTTCAGA	CCAGGTCTCA	AAGATACTAA	GCTATTGAAA	ACACTAGCTA
701	TCGGTGACAC	CATCACATCT	CAAGAATTAC	TAGCTCAAGC	ACAAAGCATT
751	TTAAACAAAA	ACCACCCAGG	CTATACGATT	TATGAACGTG	ACTCCTCAAT
801	CGTCACTCAT	GACAATGACA	TTTTCCGTAC	GATTTTACCA	ATGGATCAAG
851	AGTTTACTTA	CCGTGTTAAA	AATCGGGAAC	AAGCTTATAG	GATCAATAAA
901	AAATCTGGTC	TGAATGAAGA	AATAAACAAC	ACTGACCTGA	TCTCTGAGAA
951	ATATTACGTC	CTTAAAAAAG	GGGAAAAGCC	GTATGATCCC	TTTGATCGCA
1001	GTCACTTGAA	ACTGTTCACC	ATCAAATACG	TTGATGTCGA	TACCAACGAA
1051	TTGCTAAAAA	GTGAGCAGCT	CTTAACAGCT	AGCGAACGTA	ACTTAGACTT
1101	CAGAGATTTA	TACGATCCTC	GTGATAAGGC	TAAACTACTC	TACAACAATC
1151	TCGATGCTTT	TGGTATTATG	GACTATACCT	TAACTGGAAA	AGTAGAGGAT
1201	AATCACGATG	ACACCAACCG	TATCATAACC	GTTTATATGG	GCAAGCGACC
1251	CGAAGGAGAG	AATGCTAGCT	ATCATTTAGC	CTATGATAAA	GATCGTTATA
1301	CCGAAGAAGA	ACGAGAAGTT	TACAGCTACC	TGCGTTATAC	AGGGACACCT
1351	ATACCTGATA	ACCCTAACGA	CAAATAA		

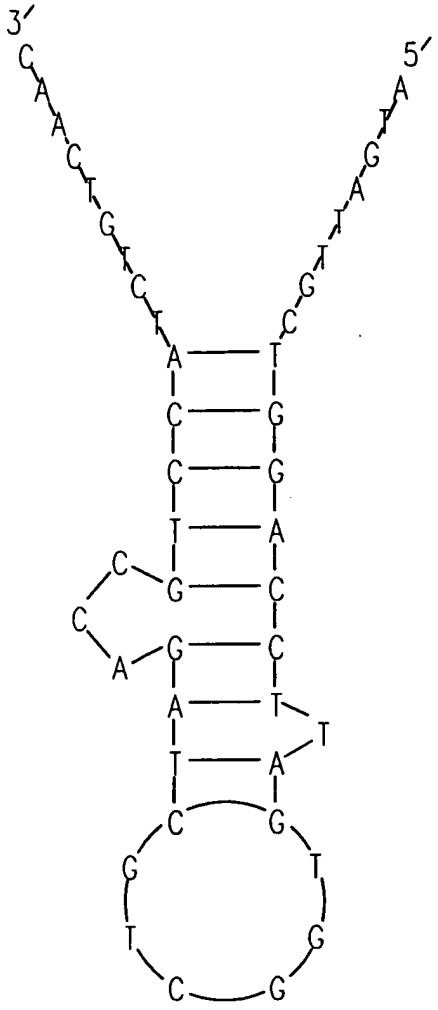


FIG. 12A

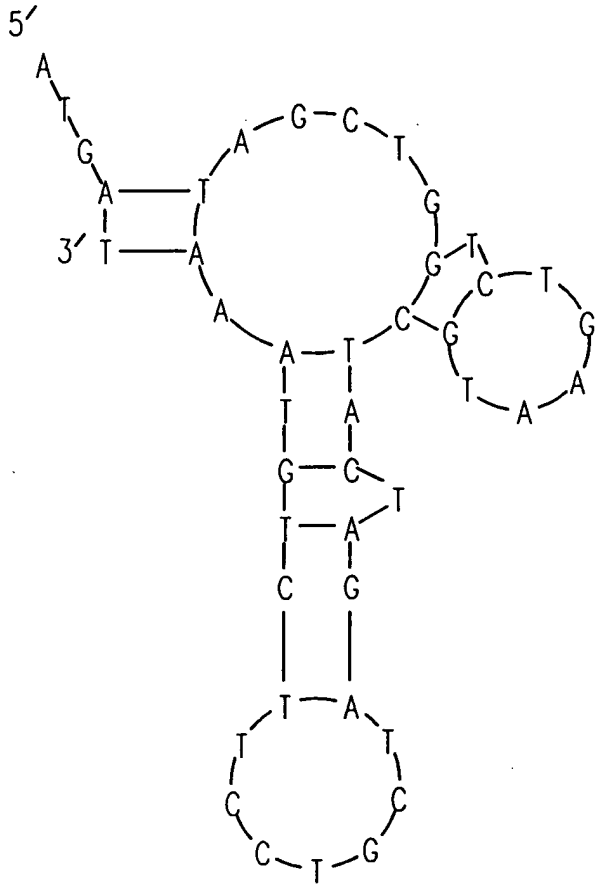


FIG. 12B

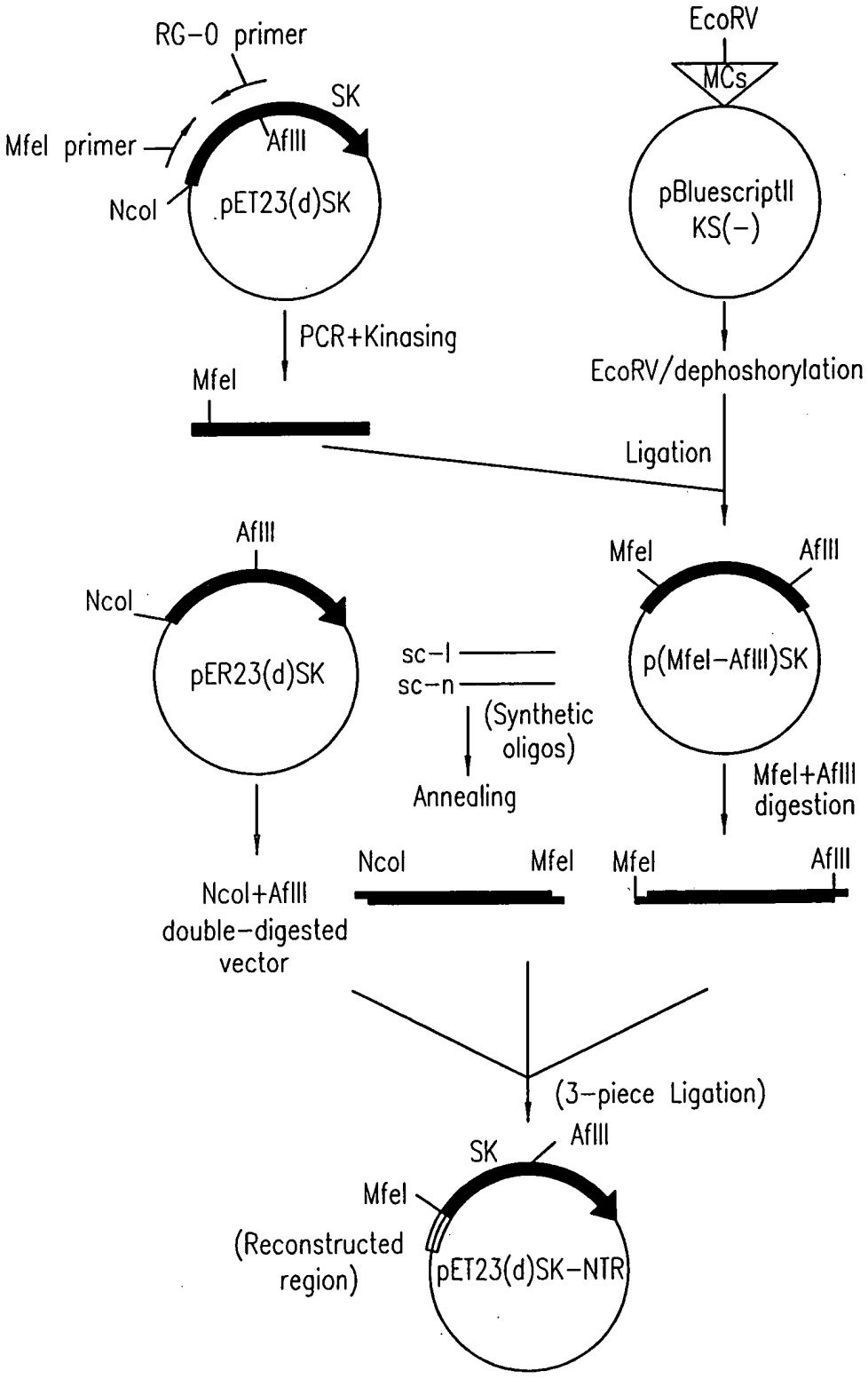

RG-0 primer
SK
MfeI primer
AflII
NcoI
pET23(d)SK
PCR+Kinasing
MfeI
EcoRV
MCs
pBluescriptII KS(-)
EcoRV/dephoshorylation
Ligation
AflII
NcoI
pER23(d)SK
sc-I
sc-n
(Synthetic oligos)
Annealing
MfeI
AflII
p(MfeI-AflII)SK
MfeI+AflII digestion
NcoI
MfeI
MfeI
AflII
NcoI+AflII double-digested vector
(3-piece Ligation)
SK
AflII
MfeI
(Reconstructed region)
pET23(d)SK-NTR

FIG. 13

FIG. 14

	10	20	30	40	50
	TAATACGACT	CACTATAGGG	AGACCACAAC	GGTTTCCCTC	TAGAAATAAT
51	TTTGTTTAAC	TTTAAGAAGG	AGATATACCA	TGATAGCTGG	TCCTGAATGG
101	CTACTAGATC	GTCCTTCTGT	AAATAACAGC	CAATTGGTTG	TTAGCGTTGC
151	TGGTACTGTT	GAGGGGACGA	ATCAAGACAT	TAGTCTTAAA	TTTTTTGAAA
201	TCGATCTAAC	ATCACGACCT	GCTCATGGAG	GAAAGACAGA	GCAAGGCTTA
251	AGTCCAAAAT	CAAAACCATT	TGCTACTGAT	AGTGGCGCGA	TGTCACATAA
301	ACTTGAGAAA	GCTGACTTAC	TAAAGGCTAT	TCAAGAACAA	TTGATCGCTA
351	ACGTCCACAG	TAACGACGAC	TACTTTGAGG	TCATTGATTT	TGCAAGCGAT
401	GCAACCATTA	CTGATCGAAA	CGGCAAGGTC	TACTTTGCTG	ACAAAGATGG
451	TTCGGTAACC	TTGCCGACCC	AACCTGTCCA	AGAATTTTTG	CTAAGCGGAC
501	ATGTGCGCGT	TAGACCATAT	AAAGAAAAAC	CAATACAAAA	CCAAGCGAAA
551	TCTGTTGATG	TGGAATATAC	TGTACAGTTT	ACTCCCTTAA	ACCCTGATGA
601	CGATTTCAGA	CCAGGTCTCA	AAGATACTAA	GCTATTGAAA	ACACTAGCTA
651	TCGGTGACAC	CATCACATCT	CAAGAATTAC	TAGCTCAAGC	ACAAAGCATT
701	TTAAACAAAA	ACCACCCAGG	CTATACGATT	TATGAACGTG	ACTCCTCAAT
751	CGTCACTCAT	GACAATGACA	TTTTCCGTAC	GATTTTACCA	ATGGATCAAG
801	AGTTTACTTA	CCGTGTTAAA	AATCGGGAAC	AAGCTTATAG	GATCAATAAA
851	AAATCTGGTC	TGAATGAAGA	AATAAACAAC	ACTGACCTGA	TCTCTGAGAA
901	ATATTACGTC	CTTAAAAAAG	GGGAAAAGCC	GTATGATCCC	TTTGATCGCA
951	GTCACTTGAA	ACTGTTCACC	ATCAAATACG	TTGATGTCGA	TACCAACGAA
1001	TTGCTAAAAA	GTGAGCAGCT	CTTAACAGCT	AGCGAACGTA	ACTTAGACTT
1051	CAGAGATTTA	TACGATCCTC	GTGATAAGGC	TAAACTACTC	TACAACAATC
1101	TCGATGCTTT	TGGTATTATG	GACTATACCT	TAACTGGAAA	AGTAGAGGAT
1151	AATCACGATG	ACACCAACCG	TATCATAACC	GTTTATATGG	GCAAGCGACC
1201	CGAAGGAGAG	AATGCTAGCT	ATCATTTAGC	CTATGATAAA	GATCGTTATA
1251	CCGAAGAAGA	ACGAGAAGTT	TACAGCTACC	TGCGTTATAC	AGGGACACCT
1301	ATACCTGATA	ACCCTAACGA	CAAATAA		

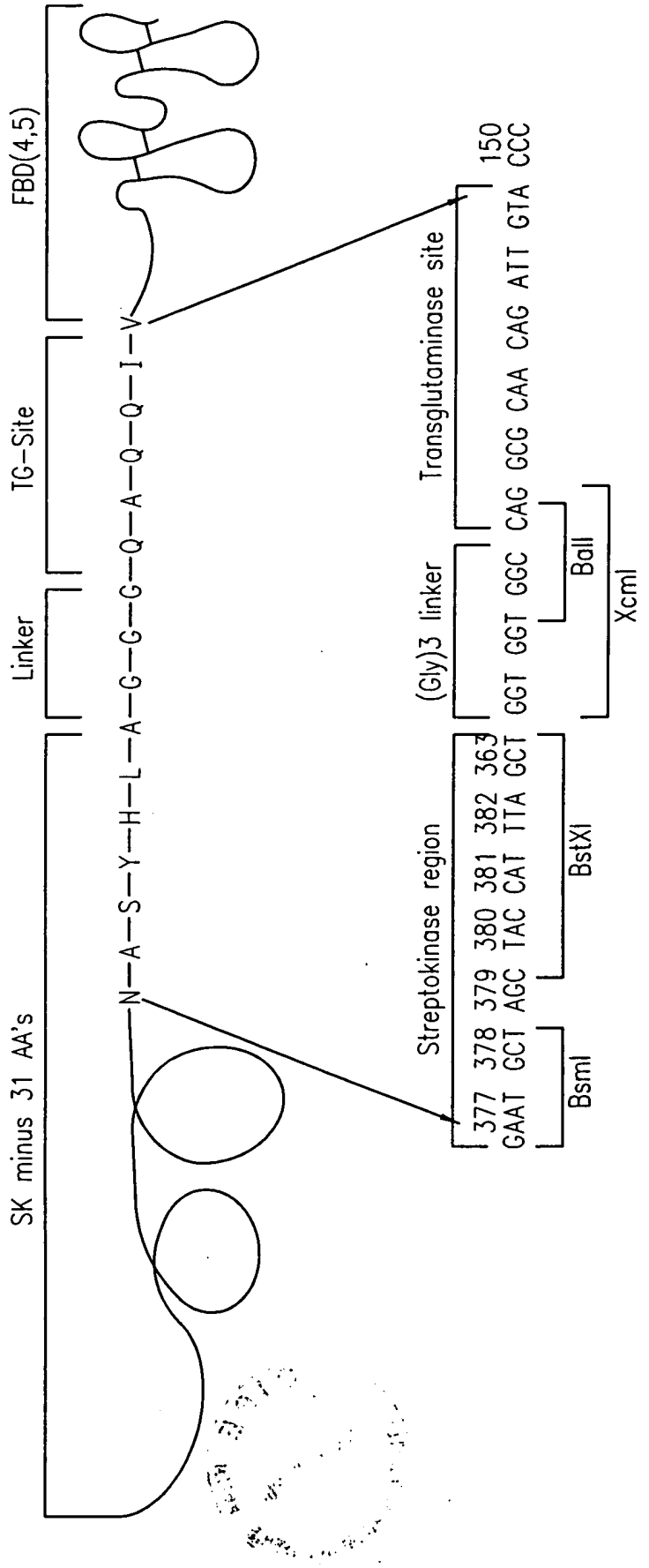

SK minus 31 AA's
Linker
TG-Site
FBD(4,5)
N—A—S—Y—H—L—A—G—G—G—Q—A—Q—Q—I—V
Streptokinase region
(Gly)3 linker
Transglutaminase site
377 378 379 380 381 382 363
GAT GCT AGC TAC CAT TTA GCT GGT GGT GGC CAG GCG CAA CAG ATT GTA CCC
150
BsmI
BstXI
BalI
XcmI

FIG. 15

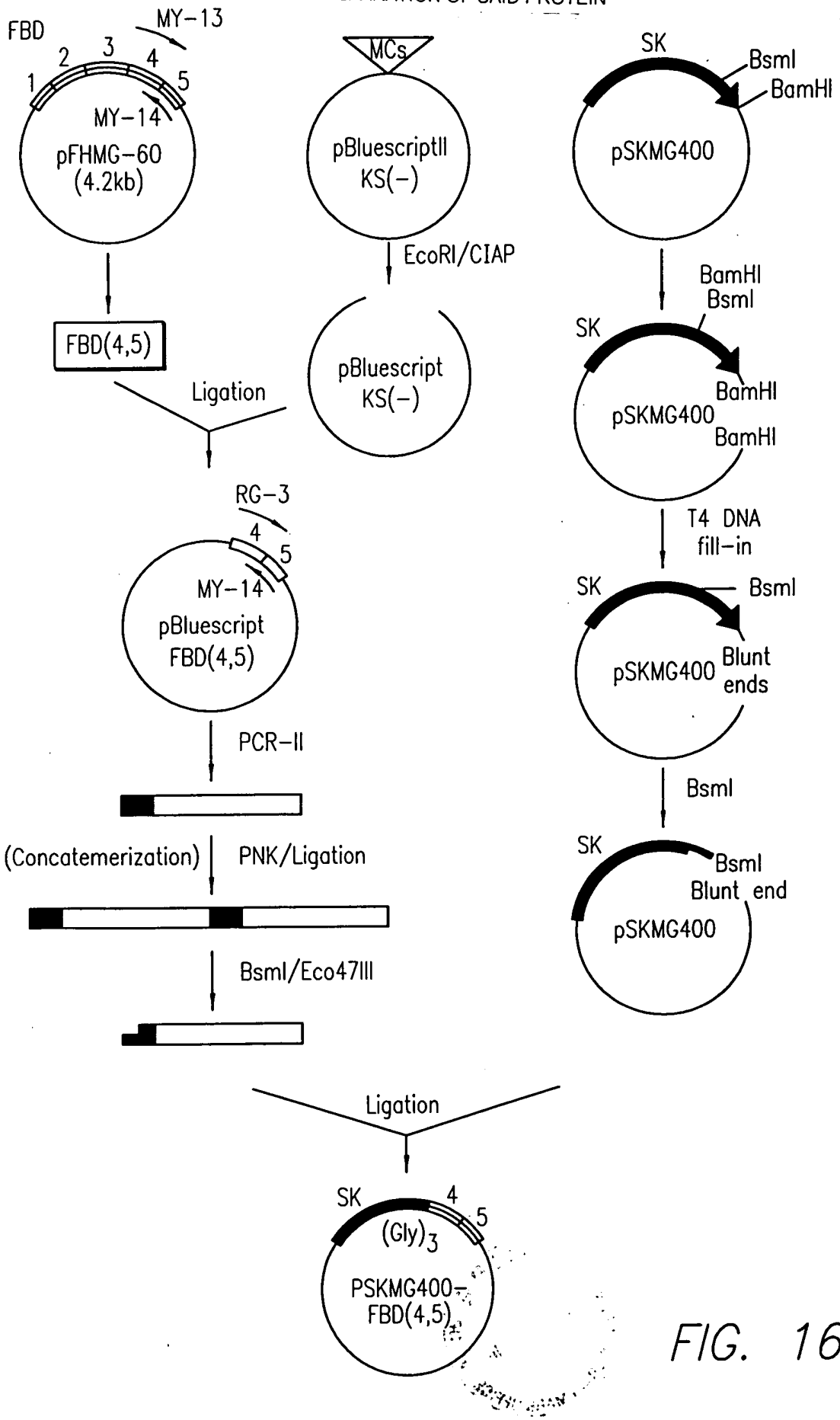

FBD
MY-13
1
2
3
4
5
MY-14
pFHMG-60
(4.2kb)
FBD(4,5)
Ligation
MCs
pBluescriptII
KS(-)
EcoRI/CIAP
pBluescript
KS(-)
SK
BsmI
BamHI
pSKMG400
BamHI
BsmI
SK
pSKMG400
BamHI
BamHI
T4 DNA
fill-in
SK
BsmI
pSKMG400
Blunt
ends
BsmI
SK
BsmI
Blunt end
pSKMG400
RG-3
4
5
MY-14
pBluescript
FBD(4,5)
PCR-II
(Concatemerization)
PNK/Ligation
BsmI/Eco47III
Ligation
SK
4
5
(Gly)3
PSKMG400-
FBD(4,5)

FIG. 16

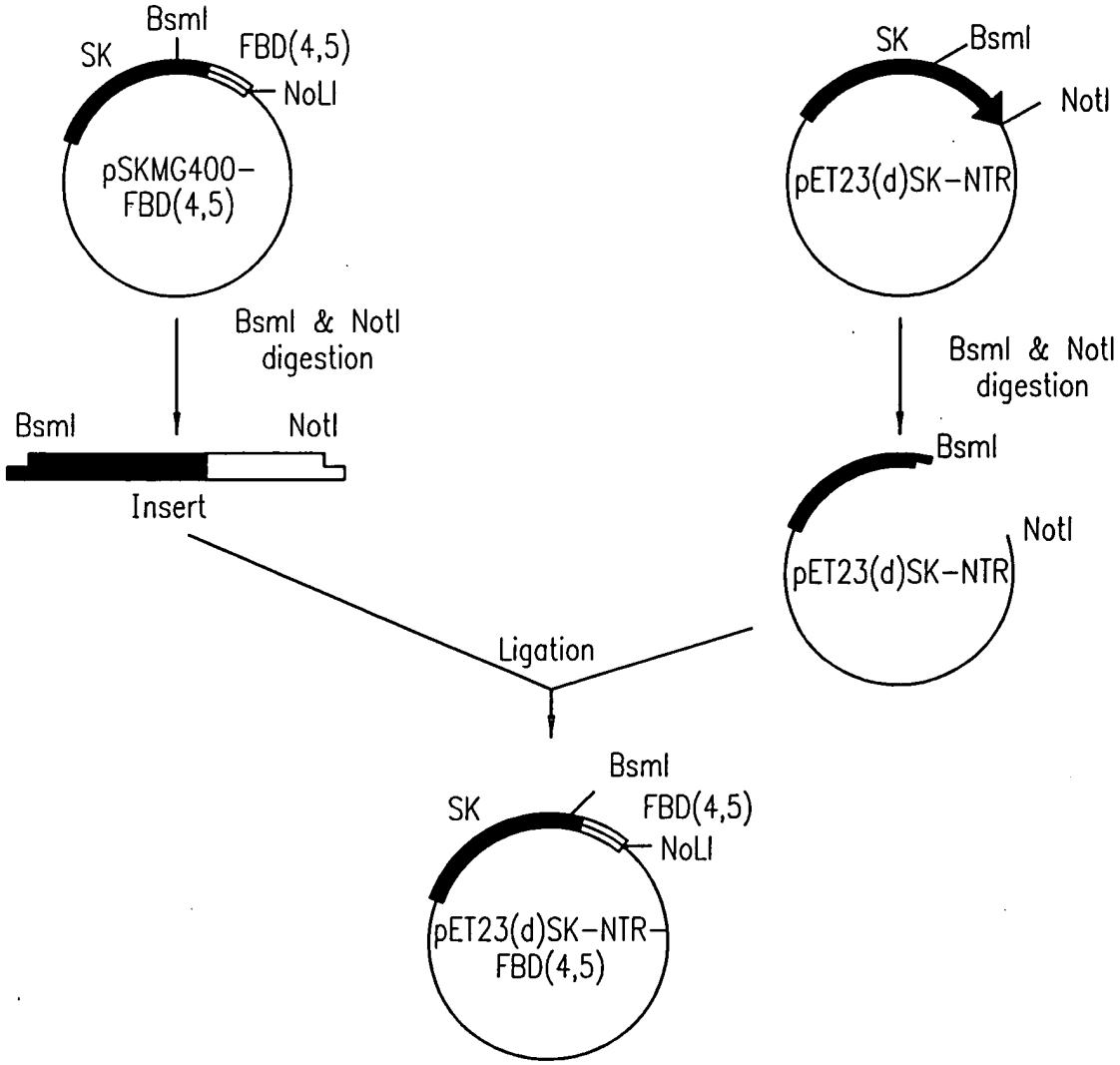

BsmI
SK
FBD(4,5)
NoLI
pSKMG400–
FBD(4,5)
BsmI & NotI
digestion
BsmI
NotI
Insert
SK
BsmI
NotI
pET23(d)SK–NTR
BsmI & NotI
digestion
BsmI
NotI
pET23(d)SK–NTR
Ligation
BsmI
SK
FBD(4,5)
NoLI
pET23(d)SK–NTR–
FBD(4,5)

FIG. 17A

FIG. 17B

	10	20	30	40	50
	TTTGTTTAAC	TTTAAGAAGG	AGATATACCA	TGATAGCTGG	TCCTGAATGG
51	CTACTAGATC	GTCCTTCTGT	AAATAACAGC	CAATTGGTTG	TTAGCGTTGC
101	TGGTACTGTT	GAGGGGACGA	ATCAAGACAT	TAGTCTTAAA	TTTTTTGAAA
151	TCGATCTAAC	ATCACGACCT	GCTCATGGAG	GAAAGACAGA	GCAAGGCTTA
201	AGTCCAAAAT	CAAAACCATT	TGCTACTGAT	AGTGGCGCGA	TGTCACATAA
251	ACTTGAGAAA	GCTGACTTAC	TAAAGGCTAT	TCAAGAACAA	TTGATCGCTA
301	ACGTCCACAG	TAACGACGAC	TACTTTGAGG	TCATTGATTT	TGCAAGCGAT
351	GCAACCATTA	CTGATCGAAA	CGGCAAGGTC	TACTTTGCTG	ACAAAGATGG
401	TTCGGTAACC	TTGCCGACCC	AACCTGTCCA	AGAATTTTTG	CTAAGCGGAC
451	ATGTGCGCGT	TAGACCATAT	AAAGAAAAAC	CAATACAAAA	CCAAGCGAAA
501	TCTGTTGATG	TGGAATATAC	TGTACAGTTT	ACTCCCTTAA	ACCCTGATGA
551	CGATTTCAGA	CCAGGTCTCA	AAGATACTAA	GCTATTGAAA	ACACTAGCTA
601	TCGGTGACAC	CATCACATCT	CAAGAATTAC	TAGCTCAAGC	ACAAAGCATT
651	TTAAACAAAA	ACCACCCAGG	CTATACGATT	TATGAACGTG	ACTCCTCAAT
701	CGTCACTCAT	GACAATGACA	TTTTCCGTAC	GATTTTACCA	ATGGATCAAG
751	AGTTTACTTA	CCGTGTTAAA	AATCGGGAAC	AAGCTTATAG	GATCAATAAA
801	AAATCTGGTC	TGAATGAAGA	AATAAACAAC	ACTGACCTGA	TCTCTGAGAA
851	ATATTACGTC	CTTAAAAAAG	GGGAAAAGCC	GTATGATCCC	TTTGATCGCA
901	GTCACTTGAA	ACTGTTCACC	ATCAAATACG	TTGATGTCGA	TACCAACGAA
951	TTGCTAAAAA	GTGAGCAGCT	CTTAACAGCT	AGCGAACGTA	ACTTAGACTT
1001	CAGAGATTTA	TACGATCCTC	GTGATAAGGC	TAAACTACTC	TACAACAATC
1051	TCGATGCTTT	TGGTATTATG	GACTATACCT	TAACTGGAAA	AGTAGAGGAT
1101	AATCACGATG	ACACCAACCG	TATCATAACC	GTTTATATGG	GCAAGCGACC
1151	CGAAGGAGAG	AATGCTAGCT	ACCATTTAGC	TGGTGGTGGC	CAGGCGCAAC
1201	AGATTGTACC	CATAGCTGAG	AAGTGTTTTG	ATCATGCTGC	TGGGACTTCC
1251	TATGTGGTCG	GAGAAACGTG	GGAGAAGCCC	TACCAAGGCT	GGATGATGGT
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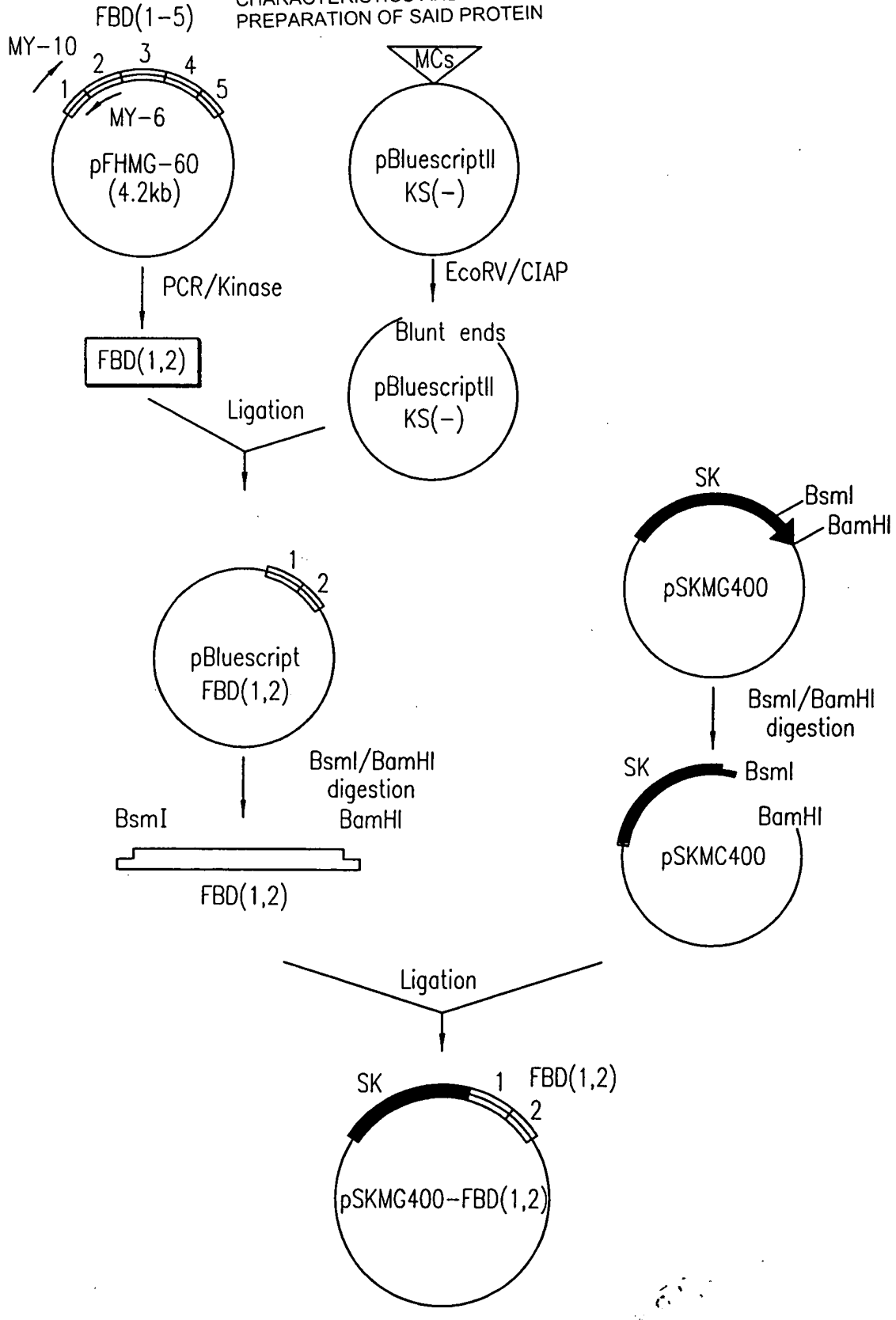
1351	GAAATAGATG	CAACGATCAG	GACACAAGGA	CATCCTATAG	AATTGGAGAC
1401	ACCTGGAGCA	AGAAGGATAA	TCGAGGAAAC	CTGCTCCAGT	GCATCTGCAC
1451	AGGCAACGGC	CGAGGAGAGT	GGAAGTGTGA	GAGGCACACC	TCTGTGCAGA
1501	CCACATCGAG	CGGATCTGGC	CCCTTCACCG	ATGTTCGTTA	G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2
3
4
5
MY-6
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(4.2kb)
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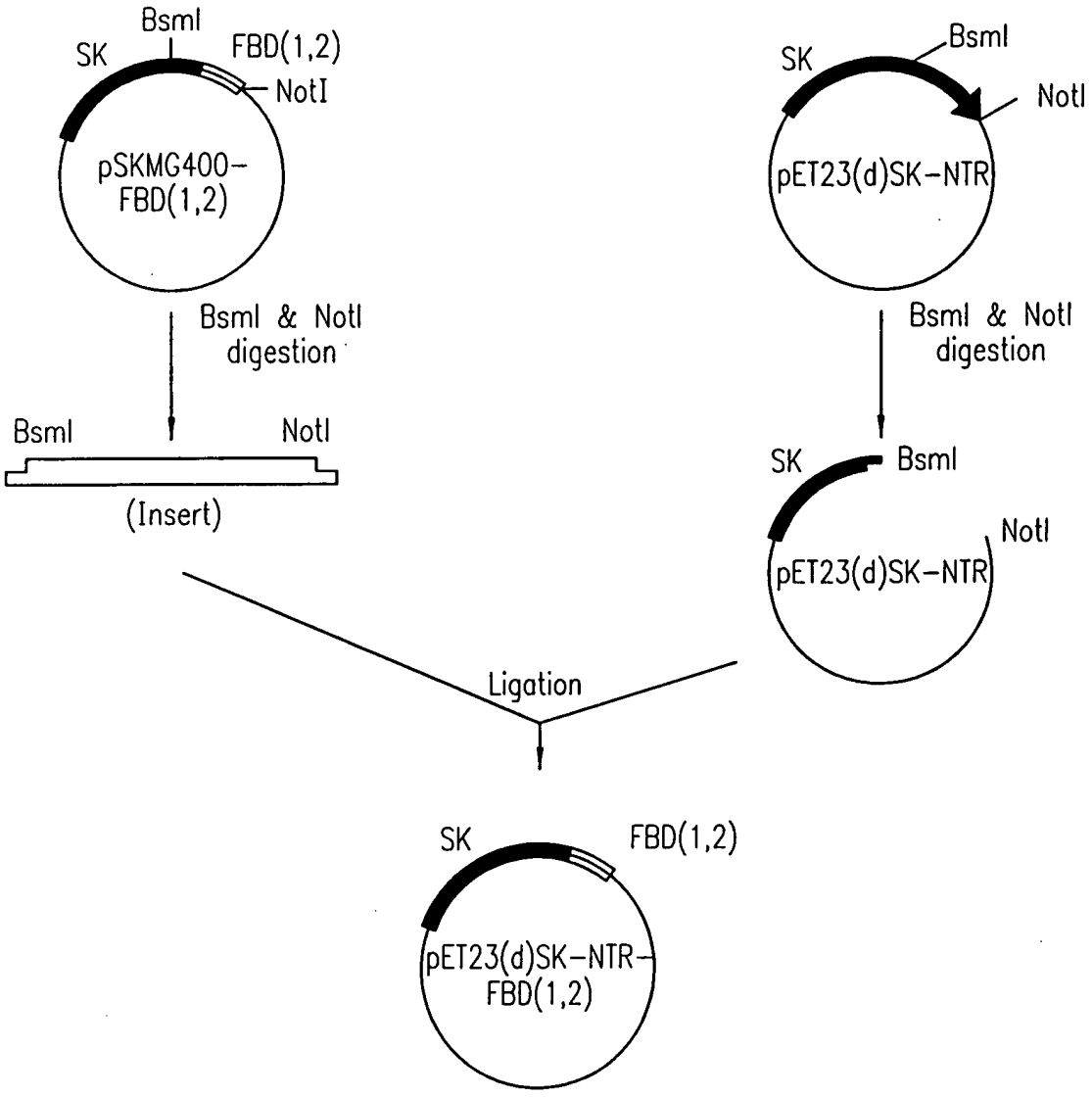
FBD(1,2)
Ligation
MCs
pBluescriptII
KS(-)
EcoRV/CIAP
Blunt ends
pBluescriptII
KS(-)
SK
BsmI
BamHI
pSKMG400
BsmI/BamHI
digestion
SK
BsmI
BamHI
pSKMC400
1
2
pBluescript
FBD(1,2)
BsmI/BamHI
digestion
BsmI
BamHI
FBD(1,2)
Ligation
SK
1
FBD(1,2)
2
pSKMG400-FBD(1,2)

FIG. 18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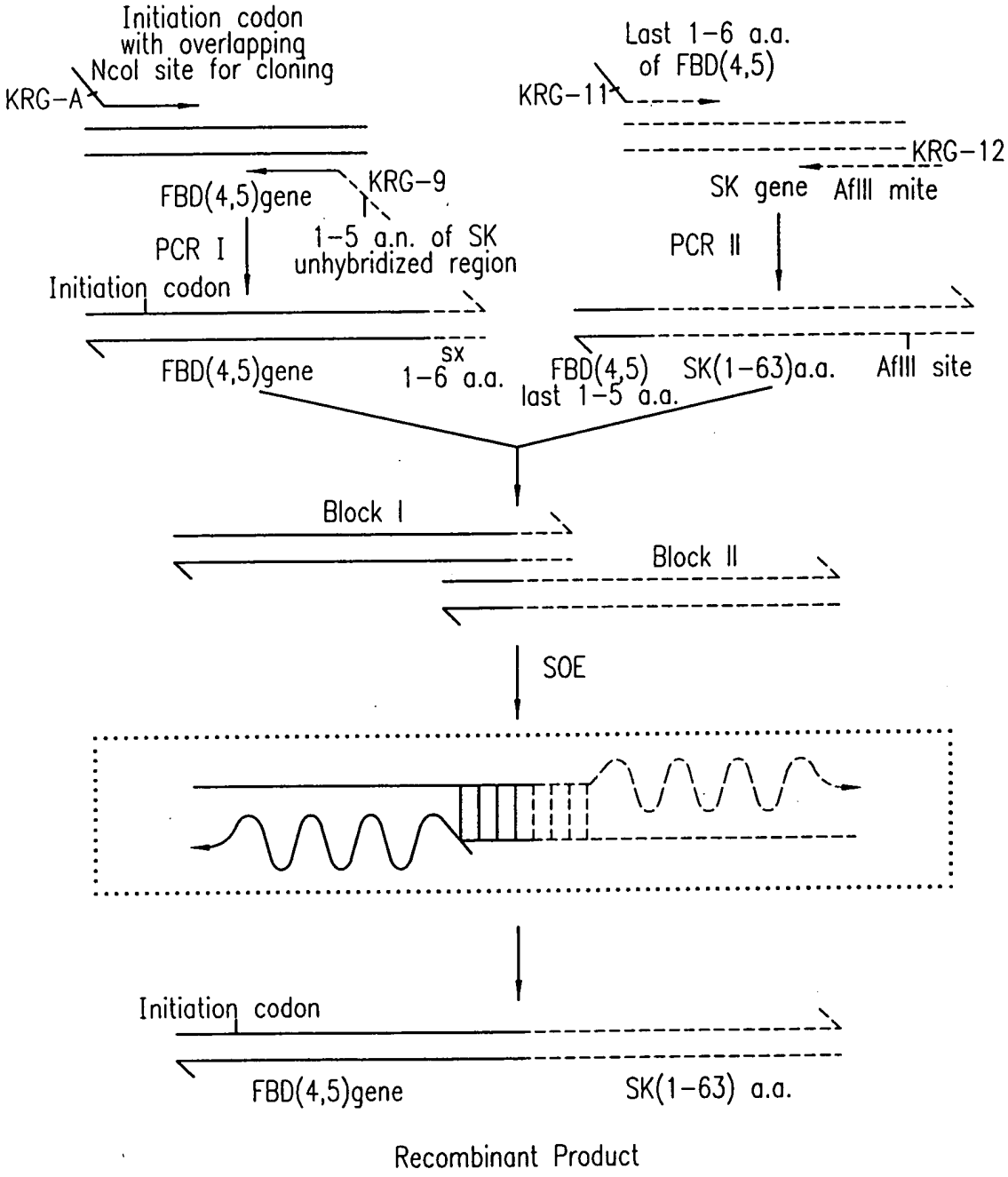
SK
BsmI
NotI
pET23(d)SK–NTR
BsmI & NotI
digestion
SK
BsmI
NotI
pET23(d)SK–NTR
Ligation
SK
FBD(1,2)
pET23(d)SK–NTR–
FBD(1,2)

FIG. 19A

FIG. 19B

	10	20	30	40	50
	GCAACCCCGC	CAGCCTAGCC	GGGTCCTCAA	CGACAGGAGC	ACGATCATGC
51	GCACCCGTGG	CCAGGACCCA	ACGCTGCCCG	AGATCTCGAT	CCCGCGAAAT
101	TAATACGACT	CACTATAGGG	AGACCACAAC	GGTTTCCCTC	TAGAAATAAT
151	TTTGTTTAAC	TTTAAGAAGG	AGATATACCA	TGATTGCTGG	ACCTGAGTGG
201	CTGCTAGACC	GTCCATCTGT	CAACAACAGC	CAATTGGTTG	TTAGCGTTGC
251	TGGTACTGTT	GAGGGGACGA	ATCAAGACAT	TAGTCTTAAA	TTTTTTGAAA
301	TCGATCTAAC	ATCACGACCT	GCTCATGGAG	GAAAGACAGA	GCAAGGCTTA
351	AGTCCAAAAT	CAAAACCATT	TGCTACTGAT	AGTGGCGCGA	TGTCACATAA
401	ACTTGAGAAA	GCTGACTTAC	TAAAGGCTAT	TCAAGAACAA	TTGATCGCTA
451	ACGTCCACAG	TAACGACGAC	TACTTTGAGG	TCATTGATTT	TGCAAGCGAT
501	GCAACCATTA	CTGATCGAAA	CGGCAAGGTC	TACTTTGCTG	ACAAAGATGG
551	TTCGGTAACC	TTGCCGACCC	AACCTGTCCA	AGAATTTTTG	CTAAGCGGAC
601	ATGTGCGCGT	TAGACCATAT	AAAGAAAAAC	CAATACAAAA	CCAAGCGAAA
651	TCTGTTGATG	TGGAATATAC	TGTACAGTTT	ACTCCCTTAA	ACCCTGATGA
701	CGATTTCAGA	CCAGGTCTCA	AAGATACTAA	GCTATTGAAA	ACACTAGCTA
751	TCGGTGACAC	CATCACATCT	CAAGAATTAC	TAGCTCAAGC	ACAAAGCATT
801	TTAAACAAAA	ACCACCCAGG	CTATACGATT	TATGAACGTG	ACTCCTCAAT
851	CGTCACTCAT	GACAATGACA	TTTTCCGTAC	GATTTTACCA	ATGGATCAAG
901	AGTTTACTTA	CCGTGTTAAA	AATCGGGAAC	AAGCTTATAG	GATCAATAAA
951	AAATCTGGTC	TGAATGAAGA	AATAAACAAC	ACTGACCTGA	TCTCTGAGAA
1001	ATATTACGTC	CTTAAAAAAG	GGGAAAAGCC	GTATGATCCC	TTTGATCGCA
1051	GTCACTTGAA	ACTGTTCACC	ATCAAATACG	TTGATGTCGA	TACCAACGAA
1101	TTGCTAAAAA	GTGAGCAGCT	CTTAACAGCT	AGCGAACGTA	ACTTAGACTT
1151	CAGAGATTTA	TACGATCCTC	GTGATAAGGC	TAAACTACTC	TACAACAATC
1201	TCGATGCTTT	TGGTATTATG	GACTATACCT	TAACTGGAAA	AGTAGAGGAT
1251	AATCACGATG	ACACCAACCG	TATCATAACC	GTTTATATGG	GCAAGCGACC
1301	CGAAGGAGAG	AATGCTAGCT	ATCATTTAGC	CGGTGGTGGT	CAGGCGCAGC
1351	AAATGGTTCA	GCCCCAGTCC	CCGGTGGCTG	TCAGTCAAAG	CAAGCCCGGT
1401	TGTTATGACA	ATGGAAAACA	CTATCAGATA	AATCAACAGT	GGGAGCGGAC
1451	CTACCTAGGT	AATGTGTTGG	TTTGTACTTG	TTATGGAGGA	AGCCGAGGTT
1501	TTAACTGCGA	AAGTAAACCT	GAAGCTGAAG	AGACTTGCTT	TGACAAGTAC
1551	ACTGGGAACA	CTTACCGAGT	GGGTGACACT	TATGAGCGTC	CTAAAGACTC
1601	CATGATCTGG	GACTGTACCT	GCATCGGGGC	TGGGCGAGGG	AGAATAAGCT
1651	GTACCATCTA	A			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KRG-9
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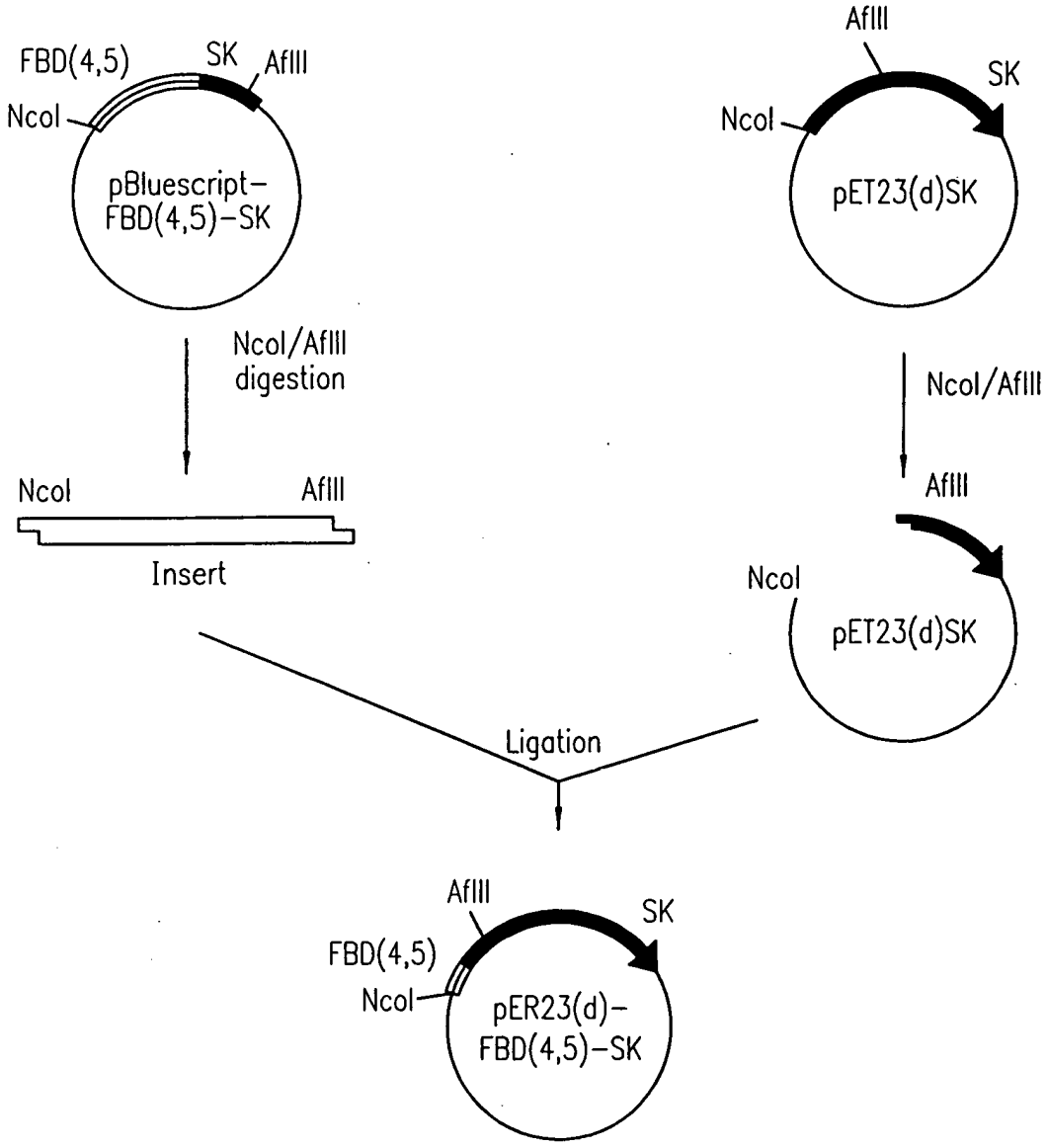
PCR I
Initiation codon
FBD(4,5)gene
SX
1-6 a.a.
Last 1-6 a.a. of FBD(4,5)
KRG-11
KRG-12
SK gene
AflII mite
PCR II
FBD(4,5) last 1-5 a.a.
SK(1-63)a.a.
AflII site
Block I
Block II
SOE
Initiation codon
FBD(4,5)gene
SK(1-63) a.a.
Recombinant Product

FIG. 20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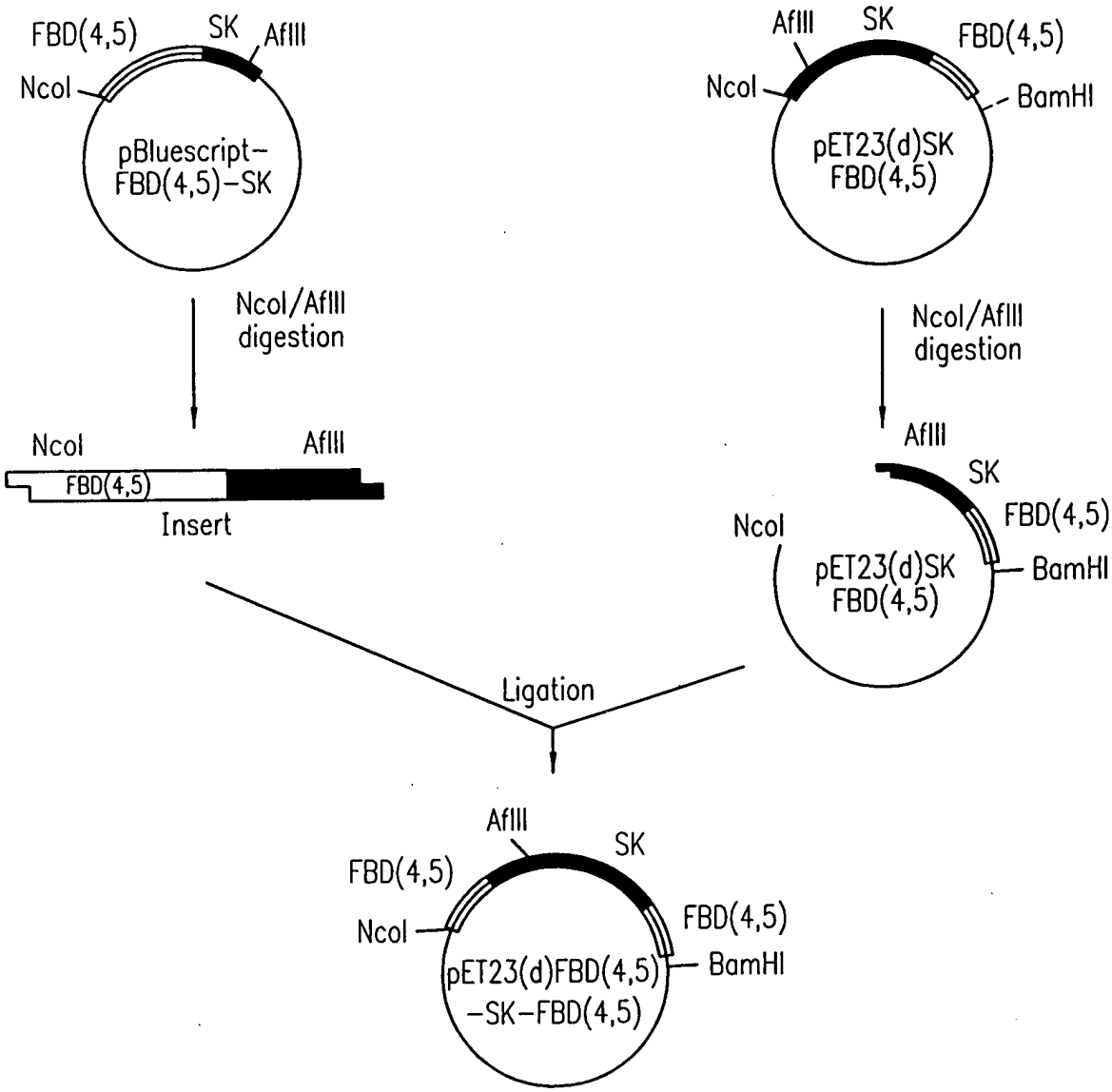
NcoI
pBluescript–
FBD(4,5)–SK
NcoI/AflII
digestion
NcoI
AflII
Insert
AflII
SK
NcoI
pET23(d)SK
NcoI/AflII
AflII
NcoI
pET23(d)SK
Ligation
AflII
SK
FBD(4,5)
NcoI
pER23(d)–
FBD(4,5)–SK

FIG. 21A

FIG. 21B

	10	20	30	40	50
1	TCGCTTCACG	TTCGCTCGCG	TATCGGTGAT	TCATTCTGCT	AACCAGTAAG
51	GCAACCCCGC	CAGCCTAGCC	GGGTCCTCAA	CGACAGGAGC	ACGATCATGC
101	GCACCCGTGG	CCAGGACCCA	ACGCTGCCCG	AGATCTCGAT	CCCGCGAAAT
151	TAATACGACT	CACTATAGGG	AGACCACAAC	GGTTTCCCTC	TAGAAATAAT
201	TTTGTTTAAC	TTTAAGAAGG	AGATATACCA	TGGTGCAAGC	ACAACAGATT
251	GTACCCATAG	CTGAGAAGTG	TTTTGATCAT	GCTGCTGGGA	CTTCCTATGT
301	GGTCGGAGAA	ACGTGGGAGA	AGGCAGCGGA	CGCATCACTT	GCACTTCTAG
351	AAATAGATGC	AACGATCAGG	ACACAAGGAC	ATCCTATAGA	ATTGGAGACA
401	CCTGGAGCAA	GAAGGATAAT	CGAGGAAACC	TGCTCCAGTG	CATCTGCACA
451	GGCAACGGCC	GAGGAGAGTG	GAAGTGTGAG	AGGCACACCT	CTGTGCAGAC
501	CACATCGAGC	GGATCTGGCC	CCTTCACCGA	TGTTCGTATT	GCTGGACCTG
551	AGTGGCTGCT	AGACCGTCCA	TCTGTCAACA	ACAGCCAATT	GGTTGTTAGC
601	GTTGCTGGTA	CTGTTGAGGG	GACGAATCAA	GACATTAGTC	TTAAATTTTT
651	TGAAATCGAT	CTAACATCAC	GACCTGCTCA	TGGAGGAAAG	ACAGAGCAAG
701	GCTTAAGTCC	AAAATCAAAA	CCATTTGCTA	CTGATAGTGG	CGCGATGTCA
751	CATAAACTTG	AGAAAGCTGA	CTTACTAAAG	GCTATTCAAG	AACAATTGAT
801	CGCTAACGTC	CACAGTAACG	ACGACTACTT	TGAGGTCATT	GATTTTGCAA
851	GCGATGCAAC	CATTACTGAT	CGAAACGGCA	AGGTCTACTT	TGCTGACAAA
901	GATGGTTCGG	TAACCTTGCC	GACCCAACCT	GTCCAAGAAT	TTTTGCTAAG
951	CGGACATGTG	CGCGTTAGAC	CATATAAAGA	AAAACCAATA	CAAAACCAAG
1001	CGAAATCTGT	TGATGTGGAA	TATACTGTAC	AGTTTACTCC	CTTAAACCCT
1051	GATGACGATT	TCAGACCAGG	TCTCAAAGAT	ACTAAGCTAT	TGAAAACACT
1101	AGCTATCGGT	GACACCATCA	CATCTCAAGA	ATTACTAGCT	CAAGCACAAA
1151	GCATTTTAAA	CAAAAACCAC	CCAGGCTATA	CGATTTATGA	ACGTGACTCC
1201	TCAATCGTCA	CTCATGACAA	TGACATTTTC	CGTACGATTT	TACCAATGGA
1251	TCAAGAGTTT	ACTTACCGTG	TTAAAAATCG	GGAACAAGCT	TATAGGATCA
1301	ATAAAAAATC	TGGTCTGAAT	GAAGAAATAA	ACAACACTGA	CCTGATCTCT
1351	GAGAAATATT	ACGTCCTTAA	AAAAGGGGAA	AAGCCGTATG	ATCCCTTTGA
1401	TCGCAGTCAC	TTGAAACTGT	TCACCATCAA	ATACGTTGAT	GTCGATACCA
1451	ACGAATTGCT	AAAAAGTGAG	CAGCTCTTAA	CAGCTAGCGA	ACGTAACTTA
1501	GACTTCAGAG	ATTTATACGA	TCCTCGTGAT	AAGGCTAAAC	TACTCTACAA
1551	CAATCTCGAT	GCTTTTGGTA	TTATGGACTA	TACCTTAACT	GGAAAAGTAG
1601	AGGATAATCA	CGATGACACC	AACCGTATCA	TAACCGTTTA	TATGGGCAAG
1651	CGACCCGAAG	GAGAGAATGC	TAGCTATCAT	TTAGCCTATG	ATAAAGATCG
1701	TTATACCGAA	GAAGAACGAG	AAGTTTACAG	CTACCTGCGT	TATACAGGGA
1751	CACCTATACC	TGATAACCCT	AACGACAAAT	AA	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AflII
NcoI
pBluescript–
FBD(4,5)–SK
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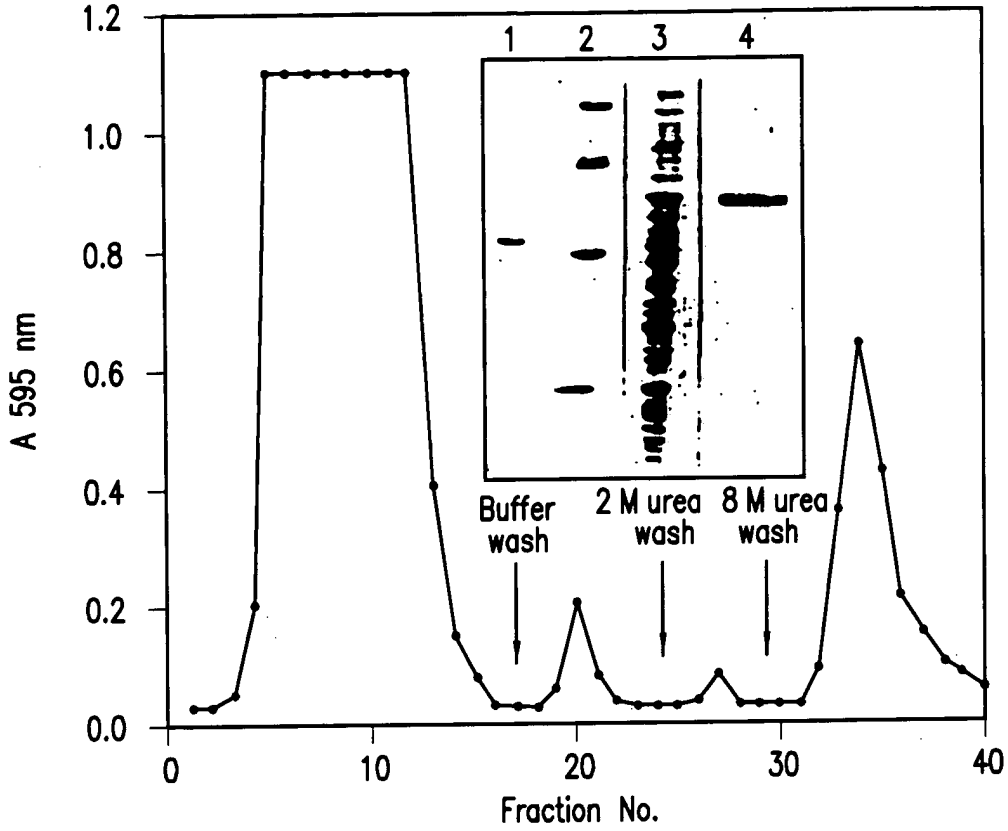
FBD(4,5)
Insert
AflII
SK
FBD(4,5)
NcoI
BamHI
pET23(d)SK
FBD(4,5)
NcoI/AflII
digestion
AflII
SK
FBD(4,5)
NcoI
BamHI
pET23(d)SK
FBD(4,5)
Ligation
AflII
SK
FBD(4,5)
FBD(4,5)
NcoI
BamHI
pET23(d)FBD(4,5)
–SK–FBD(4,5)

FIG. 22A

FIG. 22B

	10	20	30	40	50
1	CGAAGACCAT	TCATGTTGTT	GCTCAGGTCG	CAGACGTTTT	GCAGCAGCAG
51	TCGCTTCACG	TTCGCTCGCG	TATCGGTGAT	TCATTCTGCT	AACCAGTAAG
101	GCAACCCCGC	CAGCCTAGCC	GGGTCCTCAA	CGACAGGAGC	ACGATCATGC
151	GCACCCGTGG	CCAGGACCCA	ACGCTGCCCG	AGATCTCGAT	CCCGCGAAAT
201	TAATACGACT	CACTATAGGG	AGACCACAAC	GGTTTCCCTC	TAGAAATAAT
251	TTTGTTTAAC	TTTAAGAAGG	AGATATACCA	TGGTGCAAGC	ACAACAGATT
301	GTACCCATAG	CTGAGAAGTG	TTTTGATCAT	GCTGCTGGGA	CTTCCTATGT
351	GGTCGGAGAA	ACGTGGGAGA	AGGCAGCGGA	CGCATCACTT	GCACTTCTAG
401	AAATAGATGC	AACGATCAGG	ACACAAGGAC	ATTCCTATAGA	ATTGGAGACA
451	CCTGGAGCAA	GAAGGATAAT	CGAGGAAACC	TGCTCCAGTG	CATCTGCACA
501	GGCAACGGCC	GAGGAGAGTG	GAAGTGTGAG	AGGCACACCT	CTGTGCAGAC
551	CACATCGAGC	GGATCTGGCC	CCTTCACCGA	TGTTCGTATT	GCTGGACCTG
601	AGTGGCTGCT	AGACCGTCCA	TCTGTCAACA	ACAGCCAATT	GGTTGTTAGC
651	GTTGCTGGTA	CTGTTGAGGG	GACGAATCAA	GACATTAGTC	TTAAATTTTT
701	TGAAATCGAT	CTAACATCAC	GACCTGCTCA	TGGAGGAAAG	ACAGAGCAAG
751	GCTTAAGTCC	AAAATCAAAA	CCATTTGCTA	CTGATAGTGG	CGCGATGTCA
801	CATAAACTTG	AGAAAGCTGA	CTTACTAAAG	GCTATTCAAG	AACAATTGAT
851	CGCTAACGTC	CACAGTAACG	ACGACTACTT	TGAGGTCATT	GATTTTGCAA
901	GCGATGCAAC	CATTACTGAT	CGAAACGGCA	AGGTCTACTT	TGCTGACAAA
951	GATGGTTCGG	TAACCTTGCC	GACCCAACCT	GTCCAAGAAT	TTTTGCTAAG
1001	CGGACATGTG	CGCGTTAGAC	CATATAAAGA	AAAACCAATA	CAAAACCAAG
1051	CGAAATCTGT	TGATGTGGAA	TATACTGTAC	AGTTTACTCC	CTTAAACCCT
1101	GATGACGATT	TCAGACCAGG	TCTCAAAGAT	ACTAAGCTAT	TGAAAACACT
1151	AGCTATCGGT	GACACCATCA	CATCTCAAGA	ATTACTAGCT	CAAGCACAAA
1201	GCATTTTAAA	CAAAAACCAC	CCAGGCTATA	CGATTTATGA	ACGTGACTCC
1251	TCAATCGTCA	CTCATGACAA	TGACATTTTC	CGTACGATTT	TACCAATGGA
1301	TCAAGAGTTT	ACTTACCGTG	TTAAAAATCG	GGAACAAGCT	TATAGGATCA
1351	ATAAAAAATC	TGGTCTGAAT	GAAGAAATAA	ACAACACTGA	CCTGATCTCT
1401	GAGAAATATT	ACGTCCTTAA	AAAAGGGGAA	AAGCCGTATG	ATCCCTTTGA
1451	TCGCAGTCAC	TTGAAACTGT	TCACCATCAA	ATACGTTGAT	GTCGATACCA
1501	ACGAATTGCT	AAAAAGTGAG	CAGCTCTTAA	CAGCTAGCGA	ACGTAACTTA
1551	GACTTCAGAG	ATTTATACGA	TCCTCGTGAT	AAGGCTAAAC	TACTCTACAA
1601	CAATCTCGAT	GCTTTTGGTA	TTATGGACTA	TACCTTAACT	GGAAAAGTAG
1651	AGGATAATCA	CGATGACACC	AACCGTATCA	TAACCGTTTA	TATGGGCAAG
1701	CGACCCGAAG	GAGAGAATGC	TAGCTACCAT	TTAGCTGGTG	GTGGCCAGGC
1751	GCAACAGATT	GTACCCATAG	CTGAGAAGTG	TTTTGATCAT	GCTGCTGGGA
1801	CTTCCTATGT	GGTCGGAGAA	ACGTGGGAGA	AGCCCTACCA	AGCCTGGATG
1851	ATGGTAGATT	GTACTTGCCT	GGGAGAAGGC	AGCGGACGCA	TCACTTGCAC
1901	TTCTAGAAAT	AGATGCAACG	ATCAGGACAC	AAGGACATCC	TATAGAATTG
1951	GAGACACCTG	GAGCAAGAAG	GATAATCGAG	GAAACCTGCT	CCAGTGCATC
2001	TGCACAGGCA	ACGGCCGAGG	AGAGTGGAAG	TGTGAGAGGC	ACACCTCTGT
2051	GCAGACCACA	TCGAGCGGAT	CTGGCCCCTT	CACCGATGTT	CGTTAG

FIG. 23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3
4
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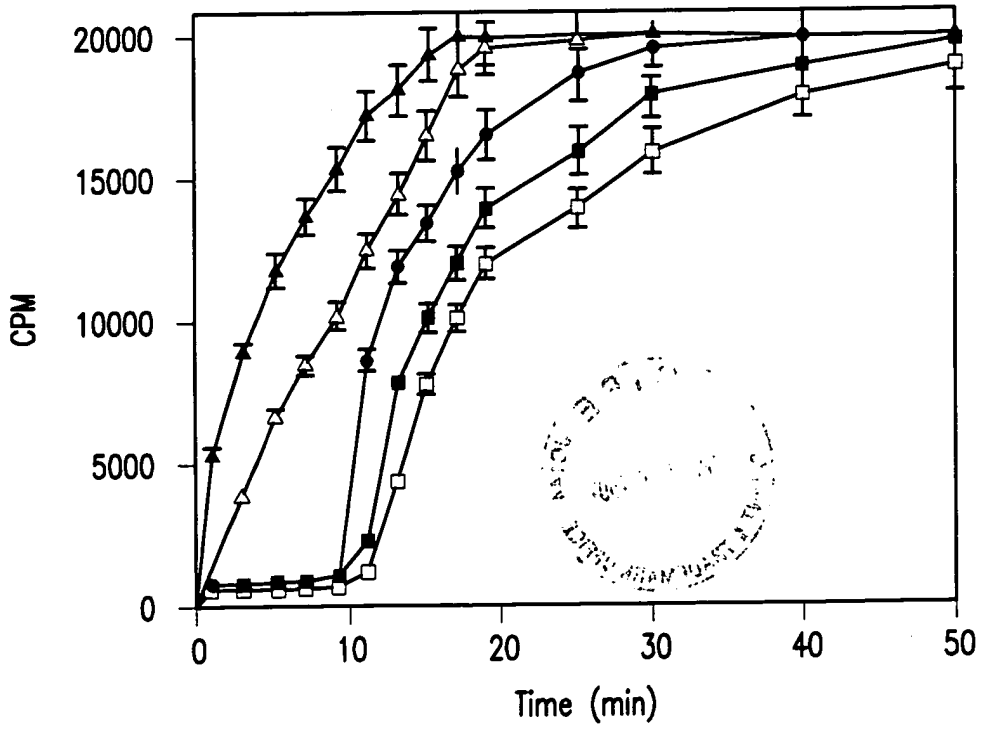
8 M urea wash

FIG. 24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